Crossability between cultivated and wild tuber-and non-tuber-bearing Solanums

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Summary

The cultivated potato has over 200 extant wild relatives many of which contain genes valuable for disease resistance, hardiness, processing and agronomic traits. Crossability of these wild species directly with the cultivated potato is complicated by several reproductive phenomena such as stylar and ploidy barriers and Endosperm Balance Numbers (EBN). However, a systematic analysis of crossability with many of these wild relatives has never fully been examined. Reciprocal crosses were made between cultivated potato and over 400 wild potato accessions; stylar barriers and 2n gamete production were examined as was the fertility of many of the putative hybrids. Generally, the seed/fruit ratio increased the more closely related the species were to the cultivated potato. However, a few crosses were successful in spite of predicted failure due to ploidy or EBN differences.

Abbreviations: CE: crossing efficiency; f: fruit; p: pollinations; pt: pollen tube growth; s: seed; s/f: seed per fruit; SG: seed germinability; %2n: 2n pollen percentage

Introduction

The cultivated potato *(Solanum tuberosum* ssp. *tuberosum)* has over 200 wild relatives found in natural populations in southwestern U.S., Mexico and Central America and following the Andes in South America (Hawkes, 1990). Wild potato species are found in a ploidy series ranging from diploid to hexaploid. The cultivated U.S. potato is tetraploid $(2n = 4\times = 48)$ and its cultivated relatives range from diploids to pentaploids. However, many of these species have interspecific crossing barriers consisting of stylar barriers, ploidy barriers and Endosperm Balance Number (EBN) incompatibilities (Hermsen, 1994; Fritz & Hanneman, 1989; Johnston et al., 1980). Except for stylar barriers, most potato species readily intercross when EBN's match, but some of the resultant progeny may exhibit low vigor or fecundity.

There are examples suggesting that pollen viability in certain interspecific hybrids is reduced or in some cases inviable (Brown, 1988; Carputo et al., 1995; Hermsen & Ramanna, 1976; Hermundstad & Peloquin, 1985; Louwes et al., 1992; Novy & Hanneman, 1991; Ramanna & Hermsen, 1974; Watanabe et al., 1995). This reduction in pollen viability was attributed to cytoplasmic-genic interactions and various meiotic abnormalities such as univalents, lagging chromosomes and arrested meioses. Female sterility has been less well documented.

There has been much study on interspecific incompatibilities among the wild species, but relatively little between the wild species and the cultivated. There are several questions that can be addressed by conducting a massive analysis of compatibility between the cultivated potato and its extant wild relatives. Do interspecific crossability barriers hold up under massive numbers of forced pollinations? Are incompatibilities

Table 1a. Series, number of species and accessions (PI's) used to represent the wild potato species

Subsection	Series[1]	# species	# PI
Estolonifera	Etuberosa	3	13
	Juglandifolia	3	9
Potatoe	Morelliformia	1	4
	Bulbocastana	4	13
	Pinnatisecta	8	34
	Polyadenia	2	8
	Commersoniana	2	10
	Circaeifolia	3	10
	Lignicaulia	1	4
	Yungasensa	4	19
	Megistacroloba	7	27
	Cuneoalata	1	5
	Conicibaccata	14	40
	Piurana	8	21
	Tuberosa (wild)	51	210
	Tuberosa (cultivated)	6	18
	Acaulia	4	21
	Longipedicellata	6	31
	Demissa	6	25
&cline3-4		134	522

[1] Series not included in study: *Olmosiana, Ingifolia* and *Maglia.*

Table 1b. Tetraploid cultivars and breeding lines used in crosses with related wild species

Variety/ Breeding line	Used as male	female
Atlantic		+
Katahdin	+	+
Kennebec	+	+
Langlade		+
Ontario	+	+
Ranger Russet	+	
Red Pontiac		+
Wischip	+	+
W AG-231	+	+
W-870		+
W-879		+
W-1005	+	+
V-122		+
10739		+
10740		+

coincidental with species boundaries and/or ploidy or EBN boundaries? Also, this work provides preliminary data for an analysis of the biosafety of transgenic potatoes in areas of natural diversity.

Materials and methods

Both wild species and cultivated varieties were analyzed in this study (Table 1a and 1b). Wild relatives of the cultivated potato were obtained from the Potato Introduction Station, NRSP-6, Sturgeon Bay, Wisconsin. Eighteen series including 134 species and subspecies represented by 522 accessions (PIs) were included. Sixteen series of subsection Potato and two series of subsection Estolonifera (Hawkes, 1990) were included in the study (Table 1a). Six to 12 plants per PI were planted in Rhinelander, where they were used as males. For use as females, eight plants were planted in the greenhouses in Madison.

The tetraploid cultivars were chosen based on male and/or female fertility (Table 1b). Several common North American cultivars were employed including Atlantic, Katahdin, Kennebec, Langlade, Ontario, Ranger Russet, Red Pontiac and Wischip.

Wisconsin advanced breeding lines used were: Wis AG 231, W-870, W-879, W-1005 and V-122. Also used were advanced lines 10740 [Ontario × (Shepody × Andigena)] and 10739 [Ontario × (Ontario × 4x hybrid)] from the USDA, ARS Potato Genetics and Enhancement Project, Madison, WI.

Crosses were made in the field at the Lelah-Starks Potato Breeding Farm, Rhinelander, Wisconsin during the summers of 1994 and 1995 and in greenhouses located at the University of Wisconsin-Madison campus in the ensuing winters. In the field, the cut-stem method was used for ease in crossing and to promote fruit retention (Hougas & Peloquin, 1959). Greenhouse crosses were made on individual potted plants. Immature flowers were emasculated, and pollen that had been collected and stored at −10 °C over anhydrous $CaCl_2$, was used. Crosses were tagged and fruit were bagged in cheesecloth. When fruit dropped (abscised) they were removed and stored at ambient temperature until the seeds could be extracted. The number of pollinations, fruit and seed were recorded. The seeds per fruit (s/f) and crossing efficiency [CE = (s/f)/pollinations] were calculated. Seed viability was determined by germinating as many as 50 seeds in soil in a greenhouse.

In crosses involving the wild species as females, styles were removed 48 to 72 hrs after pollination and placed in 3:1 (glacial acetic acid:95% ethanol) at 7 °C

until prepared for examination of pollen tube growth. Styles were stained according to Martin (1958), rinsed in dH$_2$0 and then treated for 8 to 24 hrs in 8N NaOH to soften the tissue, after which they were again rinsed in dH$_2$0 for 1 hr. Styles were placed on a slide in staining solution (0.05% aniline blue, 0.1N K$_2$PO$_4$) and squashed under a coverslip. Fluorescence of the callose was analyzed using an ultraviolet light (Zeiss HBO 5OW high pressure lamp, a G365 excitation filter with a dichroic reflector FT460, and a LP520 barrier filter). Pollen tube growth was arbitrarily measured to six regions of the style from the stigma (0) to the base of the style (6).

2n pollen measurements were determined by placing a small amount of pollen onto a glass slide in the presence of acetocarmine-glycerol and covering with a coverslip. Pollen size was analyzed with a microscope at 40X and pollen grains that were at least 1.2X larger were scored as 2n pollen. At least four fields with not less than 100 individual pollen grains were counted and averaged to determine the average percentage of 2n pollen. However, 2n pollen percentages less than 5% could be spurious due to natural variation in pollen size.

Hybridity was confirmed in the field based on morphology and/or chromosome counts.

Results

Due to the complexity and numbers of species used in the present experiment we have grouped them, for simplicity, according to Hawkes (1990). This includes, in order of putative relationship to the cultivated potato, from least to most closely related, series *Etuberosa,* Superseries *Stellata* primitive and advanced, Superseries *Rotata* primitive and advanced. We have also grouped the wild and cultivated species into separate groups.

Series Etuberosa

Males: This series has been suggested to be the link between potato and tomato (Hawkes, 1990). All of these species are 2x(1EBN) and the 2n pollen data ranged from 1–10%. *S. fernandezianum* (PI 473463) was the only successful cross, and of five seeds produced only three were viable (Table 2a). The crossing efficiency (CE) for this group was 0.003.

Females: As females, the same PI of *S. fernandezianum* was again successful setting 1 fruit and 87 seeds.

However, only 4% of these seeds were viable and the CE for the group was 1.2 (Table 2b).

The results obtained from this group are not entirely unexpected since crossability in this group to 4x(4EBN) Tuberosum cultivars is expected to be low as they are 1EBN and thus not compatible with the 4x(4EBN) cultivars. The EBN differences for the respective parents are too great to be overcome by 2n gametes, contributed by *Etuberosa* males. Additionally, there is a marked stylar incompatibility in these species when used as males. The one successful cross, 4x(4EBN) *S. tuberosum* × 2x(lEBN) *S. fernandezianum*, is anomalous with respect to the rest of this group. Data for use of these species as females is rather sparse. Only two PI's flowered enough in the greenhouse to be able to utilize them in crosses. However, it is interesting to note that the same PI, as above, of *S. fernandezianum* was again successful in producing seed and fruit. Three of the seeds from the *S. tuberosum* ssp. *tuberosum* × *S. fernandezianum* cross germinated; however, because of no tubers and flower abortion, further evaluation was not possible.

Superseries Stellata (primitive)

Series Morelliformia, Bulbocastana, Pinnatisecta and Polyadenia – Mexico

Males: These series are predominantly diploid with a few triploids, and where EBN's have been determined they are 1EBN. Also included in this group is Series *Morelliformia* which includes an epiphyte, but it never flowered sufficiently in these experiments to be included in crossing. No stylar barriers were found for these series and the 2n pollen ranged from 1–18%. Three crosses produced five fruit but no seed; therefore, the CE is 0 for this group (Table 2a).

Females: As with the males, no seed was set in any of the crosses, but unlike the males, no fruit were set either. Again the CE is 0 (Table 2b).

Series Lignicaulia, Circaeifolia and Commersoniana – South America

Males: This group is mostly 2x(1EBN) with a few triploids. Pollen tube growth was zero in the one cross measured, and 2n pollen ranged from 1–11%. Two crosses were successful in setting seed – *S. capsicibaccatum* with 25 seeds and *S. lignicaule* with 4 seeds. Only the seed from the cross involving *S. capsicibaccatum* was viable and the resultant hybrids had greater than 95% pollen sterility. They flowered prolifically

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Table 2a. Crossability of Superseries *Stellata* wild species as males with cultivated potato

		# ssp	# PI's	p	f	s	s/f	CE	% 2n	pt
Series *Etuberosa* 2x(1EBN)										
	S. etuberosum		5	86	3	0	0	0	1–8	0–6
	S fernandezianum		3	43	6	5	0.8	0.04	1–6	
	S. palustre		4	81	0	0		0	4–10	0–1
	Totals		12	210	9	5	0.6	0.003		
***Stellata* 'primitive'-Mexico**										
Series *Bulbocastana* 2x(1EBN)										
	S. bulbocastanum	3	11	201	0	0		0	2–4	6
Series *Pinnatisecta* 2x(1EBN)										
	S. brachistotrichum		3	33	0	0		0	7–13	6
	S. cardiophyllum	2	7	89	1	0	0	0	1–16	6
	S. jamesii		4	109	0	0		0	1–18	
	S. pinnatisectum		4	53	0	0		0	3–13	
	S. tarnii		4	126	0	0		0	7–13	
	S. trifidum		5	156	0	0		0	3–15	6
	subtotals		27	566	1	0	0	0		
Series *Polyadenia* 2x(?EBN)										
	S. lesteri		3	28	3	0	0	0	2–13	
	S. polyadenia		5	36	1	0	0	0	1–2	
	subtotals		8	64	4	0	0	0		
	Totals		46	831	5	0	0	0		
***Stellata* 'primitive'-South America**										
Series *Commersoniana* 2x(1EBN)										
	S. commersonii	2	10	217	3	0	0	0	1–10	0
Series *Circaeifolia* 2x(1EBN)										
	S. capsicibaccatum		3	73	1	25	25	1.39	4–7	
	S. circaeifolium	2	6	85	1	0	0	0	3–11	
	subtotal		9	158	2	25	12.5	0.08		
Series *Lignicaulia* 2x(1EBN)										
	S. lignicaule		3	53	3	4	1.3	0.05	6	
	Totals		31	428	8	29	3.6	0.01		
***Stellata* 'advanced'**										
Series *Yungasensa* 2x(2EBN)										
	S. arnezii		2	56	9	12	1.3	0.02	1–2	6
	S. chacoense		6	193	13	9	0.7	0.004	2–11	1–6
	S. huancabambense		4	92	0	0		0	4–12	6
	S. tarijense		5	116	9	13	1.4	0.01	8–20	6
	Totals	17		457	31	34	1.1	0.002		

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency; %2n = percentage 2n pollen; pt = pollen tube growth (0 = none and 6=ovaries).

and were larger than the wild species parent and set a moderate amount of tubers. Twelve crosses with these hybrids to 4x(4EBN) cultivars were attempted but none were productive. The CE for this group was 0.009 (Table 2a).

Females: This group was entirely unsuccessful in setting any seed when used as females, and the CE was zero (Table 2b).

All of the primitive *Stellata* are 2x(lEBN) species, so even the presence of 2n pollen would not be enough to overcome endosperm barriers. Few stylar barriers were detected where analyzed. As with the *Etuberosa*, these species were generally uncrossable with the cultivated potato, which due to ploidy and EBN is not unexpected. The fact that two species did produce seed when used as males was surprising. Only the

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Table 2b. Crossability of superseries *Stellata* as females with the cultivated potato

		# ssp	# PI's	p	f	s	s/f	CE
Series *Etuberosa* 2x(1EBN)								
	S *fernandezianum*		1	24	1	87	87	3.6
	S. palustre		1	47	0	0	0	0
			1	71	1	87	87	1.2
Stellata 'primitive'-Mexico								
Series *Bulbocastana* 2x(1EBN)								
	S. bulbocastanum	2	2	30	0	0	0	0
Series *Pinnatisecta* 2x(1EBN)								
	S. brachistotrichum		2	17	0	0	0	0
	S. cardiophyllum	2	4	41	0	0	0	0
	S. jamesii		2	24	0	0	0	0
	S. pinnatisectum		1	2	0	0	0	0
	S. tarnii		1	5	0	0	0	0
	S. trifidum		2	34	0	0	0	0
	subtotals	12	123	0	0	0	0	
Series *Polyadenia* 2x(?EBN)								
	S. lesteri		1	3	0	0	0	0
	S. polyadenia		2	16	0	0	0	0
	subtotals	3	19	0	0	0	0	
	Totals	17	172	0	0	0	0	
Stellata 'primitive'-South America								
Series *Commersoniana* 2x(1EBN)								
	S. commersonii	2	3	19	0	0	0	0
Series *Circaeifolia* 2x(1EBN)								
	S. capsicibaccatum		1	1	0	0	0	0
	S. circaeifolium	2	3	49	0	0	0	0
	subtotals	4	50	0	0	0	0	
Series *Lignicaulia* 2x(1EBN)								
	S. lignicaule		2	17	0	0	0	0
	Totals	9	86	0	0	0	0	
Stellata 'advanced'								
Series *Yungasensa* 2x(2EBN)								
	S. arnezii		1	43	0	0	0	0
	S. chacoense		2	39	0	0	0	0
	S. huancabambense		1	5	0	0	0	0
	S. tarijense		3	30	0	0	0	0
	Totals	7	117	0	0	0	0	

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency.

seed from the cross involving *S. capsicibaccatum* was viable and morphological analysis of the plants confirmed hybridity; however, these plants were generally of no value in further crossing schemes due to the high level of pollen inviability. Male sterility was also observed by Ramanna & Hermsen (1976) in various interspecific crosses involving these species.

Superseries Stellata (advanced)

Series *Yungasensa*
Males: Most of the species within this group are 2x(2EBN); however, a few triploids have been reported. Of the nine species within this series, four were used. The 2n pollen frequencies ranged from 1–20%.

Table 2c. Crossability between superseries *Rotata* wild species as males and the cultivated potato

		# ssp	# PI's	p	f	s	s/f	CE	% 2n	pt
Rotata 'primitive'										
Series *Cuneoalata* 2x(2EBN)										
S. infundibuliforme		4	126	0	0	0	0		5–13	5–6
Series *Megistacroloba* 2x(2EBN)										
S. astleyi		2	86	3	1	0.3	0.004		5–10	6
S. boliviense		5	135	2	0	0	0		4–10	6
S. megistacrolobum		5	225	5	0	0	0		8–16	0–6
S. raphanifolium		5	89	16	7	0.4	0.005		7–33	2–6
S. sanctae-rosae		4	165	7	4	0.6	0.003		7–11	0–6
S. sogarandinum		2	40	4	6	1.5	0.04		7–9	6
S. toralapanum		3	114	4	1	0.3	0.002		8–12	1–6
	subtotals	26	854	41	19	0.5	0.0005			
	Totals	30	980	41	19	0.5	0.0005			
Rotata 'advanced'										
Series *Conicibaccata*										
S. agrimonifolium 4x(2EBN)		2	18	0	0	0	0		10–13	
S. chomatophilum 2x(2EBN)		4	44	11	1	0.09	0.002		3–11	
S. laxissimum 2x(2EBN)		1	4	0	0	0	0		5	
S. moscopanum 6x(4EBN)		1	7	0	0	0	0			6
S. paucijugum 4x		2	39	0	0	0	0		9–12	
S. santolallae 2x		1	10	0	0	0	0			
S. tundalomense 4x		3	58	1	0	0	0		4–26	6
	subtotals	14	180	12	1	0.8	0.0005			
Series *Piurana*										
S. acroglossum 2x		1	8	0	0	0	0		1	
S. paucissectum 2x(2EBN)		1	11	0	0	0	0		6	4
S. pascoense 2x(2EBN)		1	18	0	0	0	0		2	0
S. solisii 4x		1	12	0	0	0	0			
S. tuquerrense 4x(2EBN)		5	57	0	0	0	0		4–14	1–6
	subtotals	9	106	0	0	0	0			
Series *Acaulia*										
S. acaule 4x(2EBN)	3	16	403	4	0	0	0		5–20	0–6
S. albicans 6x(4EBN)		3	34	1	1	1	0.03		5–27	6
	subtotals	19	437	5	1	0.2	0.0005			
	Totals	42	723	17	2	0.1	0.0002			
Rotata 'advanced'-Mexico										
Series *Longipedicellata* 4x(2EBN)										
S. fendleri	2	10	440	8	0	0	0		3–29	0–6
S. hjertingii		5	110	7	0	0	0		6–21	
S. matehualae		1	48	0	0	0	0		2	
S. papita		5	207	3	180	60	0.3		0–6	1–6
S. stoloniferum		5	205	3	0	0	0		5–15	0–6
	subtotals	31	1219	22	180	8.2	0.007			
Series *Demissa* 6x(4EBN)										
S. brachycarpum		5	56	0	0	0	0		6–11	0–6
S. demissum		4	166	7	42	6	0.04		3–9	5–6
S. guerreroense		2	35	2	0	0	0			
S. hougasii		4	43	0	0	0	0		3–13	0–1
S. iopetalum		5	132	5	71	14.2	0.1		2–15	0–6
S. schenckii		5	134	0	0	0	0		4–5	
	subtotals	25	566	14	113	8.1	0.01			
	Totals	56	1785	36	293	8.1	0.005			

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency; %2n = percentage 2n pollen; pt = pollen tube growth (0 = none and 6 = ovaries).

Table 2d. Crossability between superseries *Rotata* wild species as females and the cultivated potato

		# ssp	# PI's	p	f	s	s/f	CE
Rotata 'primitive'								
Series *Cuneoalata* 2x(2EBN)								
	S. infundibuliforme	1	3	0	0	0	0	
Series *Megistacroloba* 2x(2EBN)								
	S. astleyi	1	6	0	0	0	0	
	S. boliviense	2	55	0	0	0	0	
	S. megistacrolobum	2	17	0	0	0	0	
	S. raphanifolium	1	12	0	0	0	0	
	S. sanctae-rosae	2	48	0	0	0	0	
	S. toralapanum	1	25	0	0	0	0	
	subtotals	9	163	0	0	0	0	
	Totals	10	166	0	0	0	0	
Rotata 'advanced'								
Series *Conicibaccata*								
	S. agrimonifolium 4x(2EBN)	2	53	2	1	0.5	0.01	
	S. chomatophilum 2x(2EBN)	2	19	0	0	0	0	
	S. columbianum 4x(2EBN)	2	46	0	0	0	0	
	S. laxissimum 2x(2EBN)	2	38	0	0	0	0	
	S. limbaniense 2x	1	11	0	0	0	0	
	S. moscopanum 6x(4EBN)	2	34	14	504	36	1.1	
	S. paucijugum 4x	2	31	0	0	0	0	
	S. santolallae 2x	2	40	0	0	0	0	
	S. tundalomense 4x	2	43	6	385	64.2	1.5	
	S. violaceimarmoratum 2x(2EBN)	2	25	0	0	0	0	
	subtotals	19	340	22	890	40.5	0.12	
Series *Piurana*								
	S. blanco-galdosii 2x	2	22	0	0	0	0	
	S. paucissectum 2x(2EBN)	1	13	0	0	0	0	
	S. pascoense 2x(2EBN)	1	21	0	0	0	0	
	S. solisii 4x	1	24	0	0	0	0	
	S. tuquerrense 4x(2EBN)	2	21	0	0	0	0	
	subtotals	7	101	0	0	0	0	
Series *Acaulia*								
	S. acaule 4x(2EBN)	2	3	44	11	196	17.8	0.4
	S. albicans 6x(4EBN)	1	11	3	136	45	4.1	
	subtotals	4	55	14	332	23.7	0.43	
	Totals	30	496	36	1222	33.9	0.07	
Rotata 'advanced'-Mexico								
Series *Longipedicellata* 4x(2EBN)								
	S. fendleri	2	4	131	13	35	2.7	0.02
	S. hjertingii	2	37	0	0	0	0	
	S. matehualae	1	30	0	0	0	0	
	S. papita	2	27	4	2	0.5	0.03	
	S. polytrichon	2	58	1	1	1	0.03	
	S. stoloniferum	2	47	1	6	6	0.18	
	subtotals	13	330	19	44	2.3	0.007	
Series *Demissa* 6x(4EBN)								
	S. brachycarpum	2	45	20	665	33.3	0.7	
	S. demissum	2	30	9	662	73.6	2.5	
	S. guerreroense	1	23	3	93	31	1.35	
	S. hougasii	2	62	2	4	2	0.05	
	S. iopetalum	3	113	16	611	38.2	0.3	
	S. schenckii	2	39	3	11	3.7	0.09	
	subtotals	12	312	53	2046	38.6	0.1	
	Totals	25	642	72	2090	29.0	0.05	

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency.

Table 2e. Crossability between series *Tuberosa* wild species as males and the cultivated potato

Series	Species	# ssp	# PI's	p	f	s	s/f	CE	% 2n	pt
Series *Tuberosa*										
	S. abancayense 2x(2EBN)		3	129	13	8	0.6	0.005	2–19	4–6
	S. acroscopicum 2x(?EBN)		3	101	5	2	0.4	0.004	0–12	6
	S. achacachense 2x(?EBN)		1	16	1	0	0	0	5	
	S. alandiae 2x(?EBN)		5	177	17	8	0.5	0.003	0–8	6
	S. ambosinum 2x(2EBN)		4	101	9	2	0.2	0.002	2–12	6
	S. avilesii 2x(?EBN)		3	180	2	12	6	0.03	3–10	5–6
	S. berthaultii 2x(2EBN)		5	146	23	17	0.7	0.005	0–13	6
	S. brevicaule 2x(2EBN)		5	320	10	5	0.5	0.002	4–12	5–6
	S. bukasovii 2x(2EBN)		5	179	14	4	0.3	0.002	1–10	0–6
	S. canasense 2x(2EBN)		8	229	12	0	0	0	0–10	4-6
	S. candolleanum 2x(?EBN)		4	53	4	1	0.3	0.005	3–12	6
	S. chancayense 2x(1EBN)		2	25	0	0	0	0	6–17	
	S. × doddsii 2x(2EBN)		5	156	16	6	0.4	0.002	0–11	0–6
	S. dolichocremastrum 2x(?EBN)		4	66	0	0	0	0	1–4	
	S. gandarillasii 2x(2EBN)		2	31	2	0	0	0	2–10	6
	S. gourlayi 2x(2EBN)	2	15	351	9	0	0	0	2–18	1–6
	S. gourlayi 4x(4EBN)		5	146	20	310	66	2	8–16	6
	S. hondelmannii 2x(?EBN)		5	143	9	0	0	0	3–6	0–6
	S. hoopesii 4x(?EBN)		2	19	0	0	0	0	11–25	
	S. immite 2x(?EBN)		1	11	0	0	0	0	7	6
	S. incamayoense 2x(?EBN)		5	129	5	5	1	0.008	2–9	0–6
	S. kurtzianum 2x(2EBN)		1	31	0	0	0	0	6	5
	S. leptophyes 2x(2EBN)		5	151	10	3	0.3	0.002	3–9	1–6
	S. marinasense 2x(2EBN)		4	31	6	7	1.2	0.04	17–27	
	S. medians 2x(2EBN)		6	128	8	0	0	0	2–14	6
	S. microdontum 2x(2EBN)	2	6	88	9	4	0.4	0.005	4–30	6
	S. mochiquense 2x(1EBN)		3	31	0	0	0	0	0–24	
	S. multidissectum 2x(2EBN)		5	93	5	0	0	0	2–20	1–6
	S. multiinterruptum 2x(2EBN)		4	33	1	1	1	0.03	2–16	
	S. neocardenasii 2x(?EBN)		2	37	1	0	0	0	4	
	S. neorossii 2x(?EBN)		5	170	2	0	0	0	6–17	1–6
	S. okadae 2x(?EBN)		3	160	16	318	19.9	0.1	4–10	6
	S. oplocense 4x(4EBN)		5	98	13	828	220	15	5–9	6
	S. oplocense 6x(4EBN)		5	114	7	140	60	3	0–13	6
	S. pampasense 2x(2EBN)		5	108	16	404	25.3	0.2	0–21	0–5
	S. × rechei 3x(2EBN)		2	67	4	3	0.8	0.01	8–11	6
	S. scabrifolium 2x(?EBN)		1	0	0	0	0	0	4	
	S. sparsipilum 2x(2EBN)		5	154	5	140	28	0.2	6–12	5–6
	S. spegazzinii 2x(2EBN)		4	161	6	0	0	0	6–8	0–6
	S. × sucrense 4x(4EBN)		4	94	0	0	0	0	7–25	2–6
	S. ugentii 4x(?EBN)		3	90	13	918	70.6	0.8	8–13	1–6
	S. venturii 2x(2EBN)		4	151	13	0	0	0	4–19	1–6
	S. vernei 2x(2EBN)	2	8	145	24	87	3.6	0.02	2–20	1–6
	S. verrucosum 2x(2EBN)		5	147	3	0	0	0	4–11	0–2
	S. weberbaueri 2x(2EBN)		1	10	0	0	0	0	7	6
	Totals			5000	333	3233	9.7	0.002		

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency; % 2n = percentage 2n pollen; pt = pollen tube growth (0 = none and 6 = ovaries).

Table 2f. Crossability between series *Tuberosa* wild species as females and the cultivated potato

Series	Species	# ssp	# PI's	p	f	s	s/f	CE
Series *Tuberosa*								
	S. abancayense 2x(2EBN)		3	126	2	1	0.5	0.004
	S. acroscopicum 2x(?EBN)		1	2	0	0	0	0
	S. alandiae 2x(?EBN)		2	99	0	0	0	0
	S. ambosinum 2x(2EBN)		1	15	0	0	0	0
	S. andreanum 2x(?EBN)		2	71	0	0	0	0
	S. avilesii 2x(?EBN)		2	119	0	0	0	0
	S. berthaultii 2x(2EBN)		4	32	0	0	0	0
	S. brevicaule 2x(2EBN)		2	177	0	0	0	0
	S. bukasovii 2x(2EBN)		2	68	3	2	0.7	0.01
	S. canasense 2x(2EBN)		1	18	0	0	0	0
	S. candolleanum 2x(?EBN)		2	37	1	1	1	0.03
	S. chancayense 2x(1EBN)		2	58	0	0	0	0
	S. × doddsii 2x(2EBN)		1	4	0	0	0	0
	S. gandarillasii 2x(2EBN)		2	72	0	0	0	0
	S. gourlayi 2x(2EBN)	3	5	182	4	8	4	0.13
	S. gourlayi 4x(4EBN)		1	35	4	288	72	2.06
	S. hondelmannii 2x(?EBN)		2	38	1	38	38	1
	S. hoopesii 4x(?EBN)		2	107	5	147	29.4	0.3
	S. incamayoense 2x(?EBN)		2	52	0	0	0	0
	S. kurtzianum 2x(2EBN)		1	35	0	0	0	0
	S. leptophyes 2x(2EBN)		2	100	1	1	1	0.01
	S. marinasense 2x(2EBN)		2	4	0	0	0	0
	S. medians 2x(2EBN)		2	7	0	0	0	0
	S. microdontum 2x(2EBN)	2	2	16	0	0	0	0
	S. mochiquense 2x(1EBN)		1	14	0	0	0	0
	S. multidissectum 2x(2EBN)		2	125	0	0	0	0
	S. neocardenasii 2x(?EBN)		2	6	0	0	0	0
	S. neorossii 2x(?EBN)		2	127	3	2	0.7	0.005
	S. okadae 2x(?EBN)		3	157	0	0	0	0
	S. oplocense 6x(4EBN)		2	32	0	0	0	0
	S. pampasense 2x(2EBN)		5	59	0	0	0	0
	S. × rechei 3x(2EBN)		1	14	0	0	0	0
	S. sparsipilum 2x(2EBN)		2	55	0	0	0	0
	S. spegazzinii 2x(2EBN)		2	19	0	0	0	0
	S. ugentii 4x(?EBN)		2	84	1	16	16	0.2
	S. venturii 2x(2EBN)		2	62	1	1	1	0.02
	S. vernei 2x(2EBN)	2	3	11	0	0	0	0
	S. verrucosum 2x(2EBN)		2	67	0	0	0	0
	Totals			2306	26	505	19.5	0.008

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency.

Seven of the 17 accessions were successful at setting seed (41% success). Stylar barriers were assumed to be negligible as only two measurements were rated as one and the rest were six. The CE for this group was 0.002 (Table 2a) and only seed resulting from the cross of W-1005 × *S. chacoense* was germinable. Pollen viability in the hybrids was less than 70% and further crosses using these hybrids as males to 4x(4EBN) cultivars were unsuccessful.

Females: This group produced no fruit or seed when used as females in crosses with cultivars. The CE was zero (Table 2b).

Table 2g. Crossability between series *Tuberosa* cultivated species as males and the cultivated potato

Series	Species	# ssp	# PI's	p	f	s	s/f	CE	% 2n
Series	*Tuberosa*								
	S. ajanhuiri 2x(?EBN)		1	9	0	0	0	0	13
	S. × curtilobum 5x(4EBN)		3	77	15	712	47.5	0.6	3–7
	S. phureja 2x(2EBN)		4	86	2	0	0	0	3–11
	S. stenotomum 2x(2EBN)	2	7	137	18	227	12.6	0.09	1–11
	S. tuberosum 4x(4EBN)								
	ssp. *tuberosum*		4	110	14	252	18	0.16	3–10
	ssp. *andigena*		9	181	47	2432	51.7	0.29	6–31
	Totals			600	96	3623	37.7	0.06	

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency; % 2n = percentage 2n pollen.

Table 2h. Crossability between series *Tuberosa* cultivated species as females and the cultivated potato

Series	Species	# ssp	# PI's	p	f	s	s/f	CE
Series	*Tuberosa*							
	S. × curtilobum 5x(4EBN)		1	16	1	50	50	3.10
	S. phureja 2x(2EBN)	2	2	56	1	2	2	0.04
	S. stenotomum 2x(2EBN)	2	3	69	0	0	0	0
	S. tuberosum 4x(4EBN)							
	ssp. *andigena*		2	14	0	0	0	0
	Totals			155	2	52	26	0.17

Note: ssp = subspecies; PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency.

Since all of these species are 2x(2EBN), the opportunity for 2n gametes to overcome EBN barriers exists. The 2n pollen frequencies are high enough to allow for significant seed development and fruit retention. Three of four species used as males from this group were successful in setting seed; however, none were successful when used as females. This may be because fewer 2n eggs are produced than 2n pollen or that the ability to detect them is reduced. As with Hermundstad & Peloquin (1985), there was a high degree of pollen stainability in a hybrid involving *S. chacoense* suggesting that it might be reproductively viable. Pandey (1962) also reported crosses between *S. chacoense* and cultivated *S. tuberosum* to be compatible.

Superseries Rotata (primitive)

Series *Cuneoalata* and *Megistacroloba*
Males: Hawkes (1990) includes in this group portions of the series *Tuberosa* and *Conicibaccata* that are southern in distribution, but we have treated these series separately. Series *Maglia* was unable to be used in crossing because of lack of survival of transplants and/or lack of flowering. All of the species in this group are 2x(2EBN) with a few reported triploids. The 2n pollen frequencies ranged from 4–33% (Table 2c). Most of the pollen tubes grew into the ovary (6) with a few not germinating (0), although the species with no pollen germination also had accessions with pollen tubes growing into the ovaries (e.g. *S. megistacrolobum* and *S. sanctae-rosae*). Five species, represented by seven accessions, had successful seed set ranging from 0.3 to 1.5 s/f. Overall, the CE was 0.0005 and seed germination was 83%.

Only the seeds resulting from Kennebec × *S. astleyi* (PI 545848) and Kennebec × *S. raphanifolium* (PI 473369), coded J556 and J555, respectively, germinated. Only J555 survived after transplanting. Pollen analysis of J555 showed a high level (> 95%) of inviable pollen, and the viable pollen was highly heteromorphic in size. J555 was determined to be tetraploid resulting from 2n pollen. The hybrid plants produced few, small tubers and a great number of stolons, but were unsuccessful in crosses to cultivars.

Table 2i. Evaluation of hybrid progeny from potato cultivars × series *Tuberosa* wild species used as males in backcrosses to potato cultivars

Hybrid Code	Male Parent	PI	F₁ hybrid poll.[1]	stol.[2]	tub.[3]	p	f	s	s/f	CE	pt
J557	*S. abancayense*	458404		×	×						
J558	*S. alandiae*	498085	1	×	×	12	0	0	0	0	
J559	*S. ambosinum*	498209	2,5			9	1	7	7	0.78	
J562	*S. berthaultii*	498075	2,5	×	×	10	0	0	0	0	
J563	*S. bukasovii*	230506	2,6	×	×	9	0	0	0	0	
J565	*S. gourlayi*	442670	2	×	×	8	1	78	78	9.75	6
J566	*S. marinasense*	310946	2,3	×		12	2	55	27.5	2.29	
J567	*S. marinasense*	450380	2,5	×							
J568	*S. marinasense*	498254	1	×	×	11	0	0	0	0	
J569	*S. microdontum*	320307		×							
J570	*S. multiinterruptum*	275272	2,5	×	×	7	0	0	0	0	
J571	*S. okadae*	320328	2,5	×	×	12	0	0	0	0	
J573	*S. okadae*	459367	1	×	×	14	0	0	0	0	
J574	*S. okadae*	458368	1	×	×	14	1	0	0	0	
J575	*S. oplocense*	435079	2,5	×	×	7	1	20	20	2.86	
J576	*S. oplocense*	458359	2,5								
J577	*S. oplocense*	473190	1	×	×	5	0	0	0	0	6
J578	*S. pampasense*	210046	2,5	×	×	12	5	0	0	0	
J579	*S. pampasense*	275274	2,5	×	×	12	1	15	15	1.25	
J580	*S. pampasense*	275275	2,3	×	×	10	4	0	0	0	
J581	*S. pampasense*	442697	2,5		×	10	3	74	24.7	2.47	
J583	*S. pampasense*	458381	1	×	×	14	0	0	0	0	
J584	*S. sparsipilum*	310984	2,5	×	×	14	5	252	50.4	3.6	
J585	*S. sparsipilum*	473377	2,5	×		16	0	0	0	0	
J586	*S. ugentii*	546029	2,3	×		25	16	422	26.4	1.06	6
J587	*S. ugentii*	546030	2,5	×	×	12	7	1	0.1	0.01	6
J588	*S. ugentii*	546032	2,5	×	×	22	1	73	73	3.32	6
J589	*S. ugentii*	546032	2,5	×	×	22	4	252	63	2.86	6
J590	*S. vernei*	320330	1	×	×	14	5	0	0	0	
J591	*S. vernei*	473306	2,5	×		53	10	662	66.2	1.25	6
					Totals	366	67	1911	28.5	0.08	

Note: PI = plant introduction; p = pollinations; f = fruit; s = seeds; s/f = seeds per fruit; CE = crossing efficiency; pt = pollen tube growth (0 = none and 6 = ovaries).
[1] Pollen morphology based on microscopic evaluation. (1 = over 90% inviable, 2 = at least 10% viable, 3 = macropollen, 4 = micropollen and 5 = 2n pollen present).
[2] Presence or absence of stolons from field evaluation at harvest.
[3] Presence or absence of tubers from field evaluation at harvest.

Females: This group produced no fruit or seeds in crosses with the cultivars when used as females (Table 2d).

All of the species in the 'primitive' *Rotata* are 2x(2EBN) and therefore rely on 2n gametes for successful seedset with the 4x(4EBN) cultivated *Tuberosa*. There were very few stylar barriers observed, and the 2n pollen frequencies were high enough for seed set. Most of the seed from the wild species as males did not germinate, and one family

that did (Kennebec × *S. raphanifolium)* tuberized in the field but was male sterile. These species were entirely unsuccessful when used as females, probably due to the limited ability to sample 2n eggs which are fewer in number than 2n pollen.

Hermundstad & Peloquin (1985) intercrossed several species from this superseries with haploid cultivated *Tuberosa* and analyzed the fertility of 24 families produced. They suggested that an allele exists which conditions genic-cytoplasmic male sterility

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among these species. Therefore, as a whole, these species from this and previous work are generally uncrossable with the cultivated potato, and when hybrid seed is produced it has low germinability and is preponderantly male sterile.

Superseries Rotata (advanced)

Series *Piurana, Acaulia* and *Conicibaccata*

Males: Again Hawkes (1990) includes part of the series *Tuberosa* within this group, but we analyzed them separately. The species within this group span a range of ploidies and EBN's [2x(2EBN), 4x(2EBN) and 6x(4EBN)]. The majority of the pollen tube measurements were six, but there was variability both within and between species (Table 2c). 2n pollen frequencies ranged from 1 to 27%. Only two accessions representing two species were successful in setting seed, *S. chomatophilum* (PI 266387) and *S. albicans* (PI 365376) with s/f ratios of 0.09 and 1.0, respectively. *S. chomatophilum* was an interploidy, inter-EBN cross, 4x(4EBN) × 2x(2EBN), and the cross with *S. albicans* was an interploidy, intra-EBN cross, 4x(4EBN) × 6x(4EBN). Overall, the CE for this group was 0.0002. Only the seeds resulting from the cross W-1005 × *S. chomatophilum* germinated and the hybrid plant was completely male sterile, not producing any pollen.

Females: There were five successful crosses in this group when used as females, and most of them had high s/f ratios (Table 2d). Only one cross had a s/f ratio less than 17.8, that of *S. agrimonifolium*. Overall, the crosses that were fertile were highly prolific seed producers. The CE for this group was 0.07.

Series *Longipedicellata* and *Demissa* – Mexico

Males: The series *Demissa* are all 6x(4EBN) with a few pentaploids, and the series *Longipedicellata* are all 4x(2EBN). 2n pollen ranged from 0 to 29%, and all of the species had at least one accession with evidence of heteromorphic pollen (Table 2c). Twenty-one out of 56 crosses set fruit (38%), but only four of these crosses produced seed (7%). Where crosses were successful, they were very successful with moderate to high s/f ratios (Table 2c), and the overall CE for this group was 0.005.

Two of the four crosses yielded viable seed, W-1005 × *S. demissum* (PI 186551) and W-1005 × *S. iopetalum* (PI 558413), coded J550 and J551, respectively. Both of the species used were 6x(4EBN) and

therefore do not rely on 2n pollen production for seed production with the 4x(4EBN) cultivars. J550 had less than 70% stainable pollen which was highly heteromorphic with macro and micro-pollen. J551's pollen was more normal with very little inviable pollen and about 10% 2n pollen. Chromosome counts of J551 revealed it to be pentaploid, resulting from the fusion of n gametes. Both hybrids produced a few, small tubers with many stolons, and further crosses were only successful with J551, producing an average of 100.5 s/f.

Females: There were 10 successful crosses when this group was used as females, six more than as males, which is striking considering that fewer accessions were used and fewer pollinations were made. The majority of the successful crosses were with the 6x(4EBN) species (6 out of 10) (Table 2d). The s/f ratios ranged from 0.5 to 73.6, and out of 642 pollinations, 72 fruit and 2 090 seeds were produced giving a CE of 0.05. Seed germination was about 50%.

This group consists of 4x(2EBN) and 6x(4EBN) species. Higher seed set was expected in the 4EBN species due to the lack of reliance on 2n gamete production. As males, the majority of the successful crosses were with 6x(4EBN) Series *Demissa* except for the cross with 4x(2EBN) *S. fendleri* and *S. papita*. A few of the crosses exhibited some stylar barriers, from observed pollen tube growth, while other crosses to the same species exhibited no retardation of growth. Dionne (1961) observed in crosses between *S. stoloniferum* and *S. demissum* × *S. tuberosum* that there was a lack of seed set due to defective ovarian development, even though pollen tube growth and fertilization appeared normal. Hybrids from *S. tuberosum* × *S. iopetalum* were pentaploid and appeared to be male fertile based on pollen stainability. However, the hybrids from *S. tuberosum* × *S. demissum* had approximately 30% unstainable pollen, and the pollen was highly heteromorphic in size. Hybrids from both of these crosses appeared similar in the field. They were highly stoloniferous and set a few small tubers. Crossability with this group as a whole is limited by stylar barriers, EBN in the tetraploid species and some male sterility in the hybrids. Not all species are restricted by these phenomena, and thus some are readily crossable and produce fertile hybrids.

Series Tuberosa (Wild)

Males: This is the largest group of species in a single series and Hawkes (1990) divides it into three

groups based on geographical distribution. However, we chose to treat this group as one entity although analyzed by ploidy and EBN. The crossing efficiency was lower than some of the previous groups; however, most of these species are 2x(2EBN) so they rely on the production of 2n gametes to overcome EBN barriers. Pollen tube growth did not appear to be a limiting factor in most of these crosses as the pollen tubes grew down into the ovaries (Table 2e). It is interesting to note that 68% of the seed was from crosses involving 4x and 6x species which account for 10% of the total species and subspecies used. Seed germination in these crosses was 70%. 2n pollen percentages ranged from 0 to 30%. The highest s/f ratio was 70.6, and the overall CE for this group as males was 0.002.

Most of the progeny in these hybrid families followed the expected ploidies based on EBN predictions; however, there were a few notable exceptions. Some of those exceptions being hybrids with *S. alandiae*, which formed triploids and *S. vernei* hybrids which had meiotic chromosome counts ranging from 27 to 36. Hybrids of *S. ugentii*, and others, evidenced meiotic irregularities. In general, many of the crosses formed seed that was germinable and the hybrids tended to be fertile. Many hybrid families flowered and the majority of these were male fertile. There was a great deal of variation among and within families for tuberization and stolon production. Tuber size, number and shape was highly variable, but 71% of the families set tubers. The families were also variable for stolon production. Some families had few stolons while others had numerous long stolons (Table 2i).

Crosses with these hybrid families were successful. Many of the attempted crosses not only set fruit but had good seed set. The seed/fruit ratio ranged from 0 to 78 (Table 2i). Pollen tube growth, in all crosses where determined, was to the base of the style. Seed production in the successful crosses was quite good, ranging from 0 seed to 662 seeds. The seed per fruit ratios were generally high, averaging 28.5 for all the hybrid families, and 36.8 s/f for the seed producing crosses. The Crossing Efficiency of the hybrids as a whole was 0.08.

Females: As females these wild species performed better than the males with an overall CE of 0.008 (Table 2f), still less than some of the more distantly related groups though. Also, the number of seeds per fruit was 2x higher than the males (19.5 vs. 9.7). The 4x and 6x species accounted for a great percentage of the seed set, 89%, and three out the seven seed setting crosses were to the higher ploidy species. Seed germination (41%) was lower than when these species were used as males.

The wild *Tuberosa* is represented by a ploidy series, ranging from 2x(2EBN) up to 6x(4EBN). In this series, 55% of all the species used as males set seed and 26% as females. This disparity may be due to the fact that the species were used as males in cut-stem crosses and as females in on plant crosses. The cut-stem method promotes fruit retention (Hougas & Peloquin, 1959). The seeds per fruit ratio was quite high in both sets of crosses and the hybrids from the crosses using the species as males were generally quite vigorous and fertile (Table 2i). Most of the hybrid families produced stolons and tuberized and many were successful in further crosses with the tetraploid cultivars. Overall, crossability with this group was quite good with the higher seed sets being obtained with the higher ploidy (EBN) species where there was not a reliance on 2n gametes to overcome the endosperm block.

Series Tuberosa (Cultivated)

Males: These species are 2x(2EBN), 4x(4EBN) and one is 5x(4EBN). As expected, the majority of the seed set was in crosses involving the 4x(4EBN) species represented by *S. tuberosum* ssp. *tuberosum* and ssp. *andigena*. These two species accounted for 74% of the total seed produced. Surprisingly, *S. × curtilobum* [5x(4EBN)] produced 20% of the seed (Table 2g). A few diploid species did produce seed although not in the quantity that the 4x and 5x species did. 2n pollen ranged from 1 to 31% and the overall CE was 0.06. Seed germination in this group was 74%.

Females: The CE for the females was quite high, 0.17 (Table 2h); however, fewer crosses were attempted so the seed per fruit ratios are not as spectacular as with the males. Also, there was no seed set with the 4x species, although only 14 pollinations were attempted. Unexpectedly, given the high CE, the seed germination was only 2%, although most of the seed resulting from the 5x × 4x cross with *S. × curtilobum* were likely close to 4x, since *S. × curtilobum* has been maintained by sib-mating at the genebank (NRSP-6).

This series also contains diploids [2x(2EBN)] as well as polyploids [4x(4EBN)] and [5x(4EBN)]. Crossability, as determined by seed set was quite high, as expected, for this group. These species are among the most closely related and should, therefore, have some of the most prolific crosses. *S. stenotomum* was the only diploid species that set seed when used as a

male; however, it averaged 12.6 s/f. *S. × curtilobum* was the only pentaploid species (near 4x due to maintenance via sib-mating) used in these crosses, and it set seed in both directions. Other species, *S. phureja*, a diploid, produced seed when used as a female, and tetraploid *S. tuberosum* ssp. *andigena* set seed when used as a male. Previous work involving these types of crosses between the tetraploid cultivated potato and ssp. *andigena* were easily made (Hawkes, 1990). Therefore, due to the limited number of crosses made in this study, we may not have had a large enough sample size to efficiently determine the effective crossability of the tetraploid cultivated species, although, we would have expected more seed than what was obtained.

Discussion

Hawkes (1990) proposed a general scheme of series relationships that incorporates morphological and ecogeographical data. It provides a general scheme to analyze crossability data in a cohesive fashion, and with some predictability. Hawkes suggested that the corolla morphology is a defining character of the potato species and that its evolutionary change parallels that of the evolution of the species. Therefore, he proposed the two major subdivisions, superseries *Stellata* and *Rotata*, with further subdivisions within these two superseries to account for evolutionary changes and geographical location. Thus, there are four morphological, geographical divisions denoted: superseries *Rotata* 'primitive and advanced' and the superseries *Stellata*, 'primitive and advanced'. There is a general trend for ploidy and EBN to increase from the primitive *Stellata* [2x(1EBN)] to the advanced *Rotata* [4x(2EBN & 4EBN) and 6x(4EBN)]; hence, it can be expected that crossability should increase as ploidy and EBN differences are eliminated or reduced.

Crossability, by superseries and series exhibits several interesting characteristics. First the aberrant cross with series *Etuberosa* – the cross with *S. fernandezianum* yielded one fruit and 87 seeds. This result is uncharacteristic of other results with the *Etuberosa* in this study and others. If this cross is discounted in the study as aberrant, probably not a true hybrid, and therefore not representative of the series as a whole, the crossability with the *Etuberosa* is quite low. Secondly, crossability shows a general increase in seed production and 'Crossing Efficiency' as the proposed relatedness to the cultivated Solanums increases

(Table 3). It is interesting to note the preponderance of successful crosses in the more unrelated species when the species are used as males. It is also interesting that in the 'cultivated' *Tuberosa,* the species when used as males performed better than the females in the s/f ratios (Table 3). However, the situation is reversed for the CE where it is higher for the females than the males. Therefore, fewer pollinations are required for the 'cultivated' *Tuberosa* to achieve its s/f ratio as females than as males. So while high s/f ratios are achievable in either direction, they are easier to obtain when the 'cultivated' *Tuberosa* species are used as females. It is obvious from Table 3 that the majority of the successful crosses were with those species that were more closely related to the cultivars.

Analysis of CE trends across superseries and series is informative. CE's take seed/fruit ratios and make them meaningful by giving a ratio to the number of pollinations required to get a s/f ratio. This takes out some of the bias introduced by getting a large number of seeds from a few fruit, but requiring a great number of pollinations to get one or more fruit. Seed/fruit ratios are thought to remove most of the environmental variance in determining crossability, whereas pollinations include some of the environmental variance. Therefore, some series that looked quite good in s/f ratios as males, such as the 'advanced' *Rotata* and the wild *Tuberosa*, actually have CE statistics that are less striking (Table 3). Thus, the inherent crossability of these groups is less than is expected from simple seed/fruit ratios. The species, when used as females, are still quite good parents with the 4x cultivars. The 'advanced' *Rotata*, the *Conicibaccata* and the cultivated *Tuberosa* are quite effective at producing good s/f ratios with fewer requisite pollination attempts than are the other superseries and series.

Another analysis of crossability that neglects species relationships and ecogeography, but accounts for ploidy and EBN barriers, is to analyze overall crossability entirely by ploidy and EBN. Expectations would suggest that seed/fruit ratios and CEs should increase, in a linear fashion, as ploidy and EBN of the wild species increases. Therefore the lowest successful crossability should be relegated to the 2x(1EBN) species, while the 4x(4EBN) and 6x(4EBN) species should have the highest crossability.

As expected, there is a trend for increased seed/fruit and CE ratios as ploidy and EBN increase (Figures 1 and 2). The majority of the successful crosses were with the tetraploids, pentaploids and hexaploids. This is to be expected as the ploidy block

Table 3. Crossability Efficiencies (CE), seeds/fruit (S/F) and seed germinability (SG) grouped by superseries in crosses with 4x(4EBN) *S. tuberosum* ssp. *tuberosum* cultivars

Superseries/Series	CE[1]		S/F[2]		SG[3]	
	male	female	male	female	male	female
Etuberosa	0.003	1.2	0.6	87.0	60	4
Stellata 'primitive' Mexico	0	0	0.0	0		
Stellata 'primitive' South America	0.009	0	3.6	0	72	
Stellata 'advanced'	0.002	0	1.1	0	38	
Rotata 'primitive'	0.0005	0	0.5	0	83	
Rotata 'advanced' South America	0.0002	0.07	0.1	33.9	100	9
Rotata 'advanced' Mexico	0.005	0.05	8.1	29.0	75	50
Tuberosa 'wild'	0.002	0.008	9.7	19.4	70	41
Tuberosa 'cultivated'	0.06	0.2	37.7	26.0	74	2

[1] Crossing Efficiency [(s/f)/p].
[2] Seeds per fruit.
[3] Seed germinability given as a percentage.
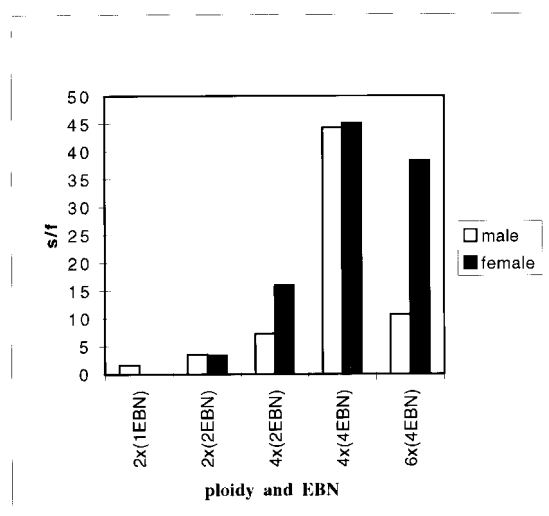
Figure 1. Crossability, as given by the seeds/fruit (s/f) ratio, between the cultivated *S. tuberosum* ssp. *tuberosum* and related wild tuber-bearing and non-tuber-bearing species analyzed by ploidy and EBN.
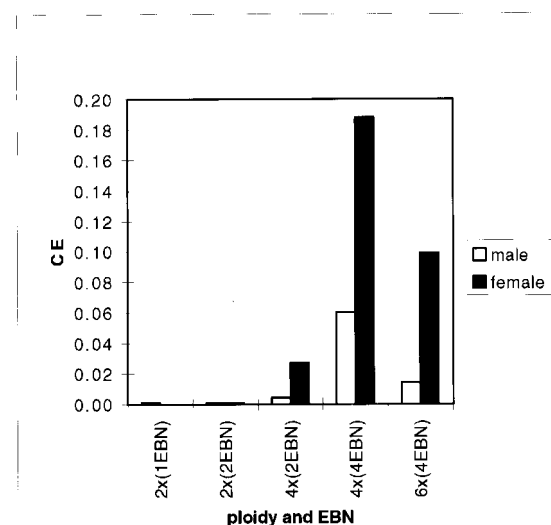

Figure 2. Crossability, as given by the crossing efficiency (CE) ratio, between the cultivated *S. tuberosum* ssp. *tuberosum* and related wild tuber-bearing and non- tuber-bearing species analyzed by ploidy and EBN.

is reduced or removed by increasing ploidy of the wild species parent in crosses with 4x(4EBN) cultivated *S. tuberosum.* Generally the successful crosses were 2EBN or 4EBN. This is also expected based on previous work which demonstrates that crossability between 4EBN species should succeed, and crossability between 4EBN and 2EBN species can succeed in the presence of 2n gametes (den Nijs & Peloquin, 1977).

Most crosses follow expectations derived from ploidy and EBN theory. However, there are crosses that somehow overcome the barriers and produce seed.

The analysis of 2n pollen among the representative wild species revealed significant levels of seed production in almost every species, and certainly in every series. The implications of polyploid evolution via the production of 2n gametes has been discussed by den Nijs & Peloquin (1977). However, the distribution of 2n pollen among so many species can only lend credence to the theory of polyploid evolution via 2n gametes. Certainly the presence of 2n pollen throughout the wild species increases the potential of

hybridization with 4x(4EBN) *S. tuberosum* for those species that would not normally hybridize due to EBN (Ehlenfeldt & Hanneman, 1984). This expands the gene pool for breeding purposes and also increases the potential for gene flow in areas of cultivation sympatric to wild species.

The reoccurring theme of hybrid sterility does raise concerns as to the role of some of these species in breeding programs and has implications for the spread of new hybrids and related gene flow. Certainly many of the hybrids generated in crosses with the wild species that are proposed to be more distantly related (Hawkes, 1990) had moderate to extreme levels of male sterility, such as the hybrids generated from crosses with *Stellata* 'primitive and advanced' and the 'primitive' *Rotata*. Another complication is the lack of germinability of seeds in many of the crosses to the *Stellata* 'primitive and advanced', *Etuberosa* and 'primitive' *Rotata*.

To incorporate this germplasm into a breeding program the following considerations are necessary. First, do any stylar barriers exist between these parents? It is important to consider reciprocal cross combinations as some of the species, especially the self-compatible ones, exhibit a unilateral incompatibility with self-incompatible species such as the cultivated 4x(4EBN) cultivars (Grun & Aubertin, 1966). Secondly, do the EBNs match or are 2n gametes needed to overcome the EBN barrier (Ehlenfeldt & Hanneman, 1984; Johnston et al., 1980; Johnston & Hanneman, 1982). The examination of the wild species for 2n gametes should help in choosing parents, as will the crossing data from 2EBN × 4EBN crosses. Thirdly, will there be sterility among the generated hybrids? Hermunstad & Peloquin (1985) determined in several species × haploid *Tuberosum* crosses that there was a genic-cytoplasmic sterility in the hybrid dependent on the genetic contribution of the species. In this study, there was sterility in certain hybrid families, but it is uncertain whether it was genic-cytoplasmic.

Crossability between the wild species and the cultivated *S. tuberosum* ssp. *tuberosum* yielded few surprises. Generally, crossability increased as proposed evolutionary distance decreased. Crossability of the species increased as ploidy and EBN increased (Figures 1 and 2). There were a few unexpectedly prolific crosses, such as that with *S. fernandezianum*. The predictiveness of this study suggests that the majority of the seed producing crosses, and the crosses with the greatest efficiency, lie in the wild and cultivated *Tuberosa*, the series *Conicibaccata* and the superseries

Rotata 'advanced'. Therefore one can use this predictiveness to choose parents in a crossing scheme, or to analyze potential gene flow in natural populations. There is the potential of introducing wild germplasm directly into a cultivated background from species that were previously not thought to be directly crossable. Several of the 2x(1EBN) species unexpectedly produced seed in crosses with the 4x(4EBN) cultivars. If seed germinability is somehow increased in these hybrids, this may potentially open the door to new and desirable, previously untapped traits. Direct crossability of wild species with the cultivated potato means that breeders may be able to shorten the route by which wild germplasm is incorporated into cultivated background.

Acknowledgement

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